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REVIEW

Homeostatic Integration of Stem Cell Dynamics during Palleal Budding of Ascidians

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ABSTRACT—A palleal bud of botryllid and polystyelid ascidians consists of a double-walled vesicle, of which the inner epithelium and hemoblasts in the blood are stem cells that play a key role in bud morphogenesis. Morphogenesis begins with the formation of pharyngeal and gut rudiments that define the future anterior and posterior ends of the body, respectively. In *Polyandrocarpa misakiensis,* it follows the mitotic cell activation of both kinds of stem cells and the epithelial transformation of hemoblasts. Those behaviors of stem cells are influenced both by humoral factor(s) from the parent and by short- and long-range cell signallings based on parental positional information, enabling homeostatic integration of primary body patterning of buds. Such morphogenetic events involve granular exocytosis of bioactive substances such as a galactose-binding, 14kDa lectin, that forms the extracellular matrix in the mesenchymal space during the earliest stage of bud development. We suggest that in budding of ascidians homeostatic cell and tissue interactions for pattern formation depend partially, at least, on the spatio-temporally regulated exocytosis of so-called autacoids.

INTRODUCTION

Cell and tissue homeostasis is one of the most important subjects of study in developmental biology. It governs dynamic equiliblium of cell number in many tissues other than static cell population such as nerve and skeletal muscle [for review, 1, 2]. In the epidermis, for example, stem cells of keratinocytes divide in the basal layer and migrate upward, and finally, squames are shed continuously from the surface of the epidermis [3, 4]. The rates of cell proliferation and cell loss must be equal, as the epidermis remains constant in thickness. Without this equiliblium, it is impossible to maintain tissue archtecture and function.

Homeostatic equiliblium is broken down temporarily in the process of regeneration. Partial hepatectomy triggers cell division of, mainly, parenchymal cells, known as compensatory hyperplasia [for review, 5]. Regenerates seem to undergo *de novo* homeostatic integration soon after the onset of regeneration in order to construct structures in the adequate position and proportion. In hydras, regenerating head blocks the additional formation of heads, referred to as lateral inhibition [6–8]. It is somewhat ambigious whether lateral inhibition, or homeostatic cell interacations, governs embryonic development. But, in the valva's equivalence group of *Caenorhabditis elegans* is there increasing evidence that the fate of each precursor cell depends on a combination of two intercellular signals, one is inductive and the other is inhibitory [e.g. 9, 10].

In this article, we review homeostatic cell interactions during budding and primary body patterning of some botryllid and polystyelid ascidians. Budding as such is a kind of regeneration, as it involves the reconstruction of adult organization from a part of parental tissues. In the first part of this article, we review the basic strategy of blastogenesis in ascidians. Special attention is paid to the behaviors of epithelial and hemopoietic stem

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cells during primary body patterning. Second, we introduce several experiments, using *Polyandrocarpa misakiensis,* that have shown the manner by which so-called positional information influences those behaviors of stem cells and the body patterning of buds. Last, the effector molecules that might govern the coordinated behavior of stem cells during bud development is discussed with reference to spatio-temporal regulation of granule exocytosis.

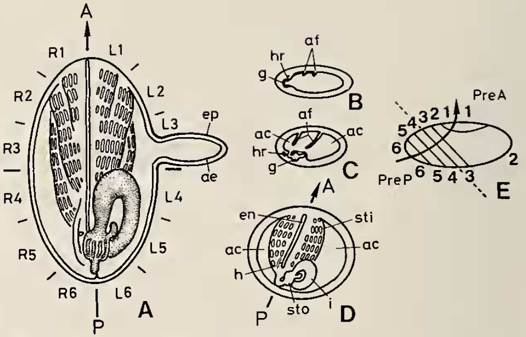

Fig. 1. Semidiagrammatic representation of body organization of an adult animal, palleal budding and antero-posterior body patterning of a bud in *Polyandrocarpa misakiensis.* (A) Each side of the adult animal is divided proportionally into six sectors, ventral view. A bud arises at any sector around the basal margin, and is endowed with the parental epidermis (ep) and atrial epithelium (ae), between which are there blood cells. (B)–(D) Morphogenetic events of the bud are usually restricted to the proximal end of the bud's proximal-distal axis (P→D). The gut rudiment (g) develops into the stomach (sto), pyloric caecum (pc) and intestine (i). The pharynx forms as atrial folds (af), being separated from the atrial chamber (ac). It is elaborated to form the stigmata (sti) and endostyle (en). The heart is derived from a small cell aggregate, heart rudiment (hr), in the mesenchymal space. The antero-posterior axis (A←P) is thus established. (E) Antero-posterior fate map of a bud obtained by chimera experiment (see the text). The axis (arrow) is skewed toward the parental anterior end. The broken line separates the bud into a presumptive anterior half (pre A) and a presumptive posterior half (pre P). From Kawamura [42].

BASIC STRATEGY OF BLASTOGENESIS IN BOTRYLLIDS AND POLYSTYELIDS

(1) *Epithelial and hemocoelomic stem cells*

A palleal bud consists of outer and inner epithelia which are, respectively, derived from the epidermis and atrial epithelium, between which are there mesenchymal cells (Fig. 1). Histological description of bud formation and bud development has been given in *Botryllus* [11, 12], *Botrylloides* [13], *Symplegma* [14–16], *Metandrocarpa* [17] and *Polyzoa* [18, 19] for botryllid ascidians and *Stolonica* [20], *Distomus* [20] and *Polyandrocarpa* [21–23] for polystyelid ascidians. In *Botrylloides, Symplegma, Polyzoa* and *Polyandrocarpa* mitotic figures in a growing bud were distributed randomly on its outer and inner epithelia (unpubl. data, see Fig. 2), showing that there is no particular meristem such as progress zone of avian limb bud [24]. Mitotic indices of the inner epithelium were 2.0–

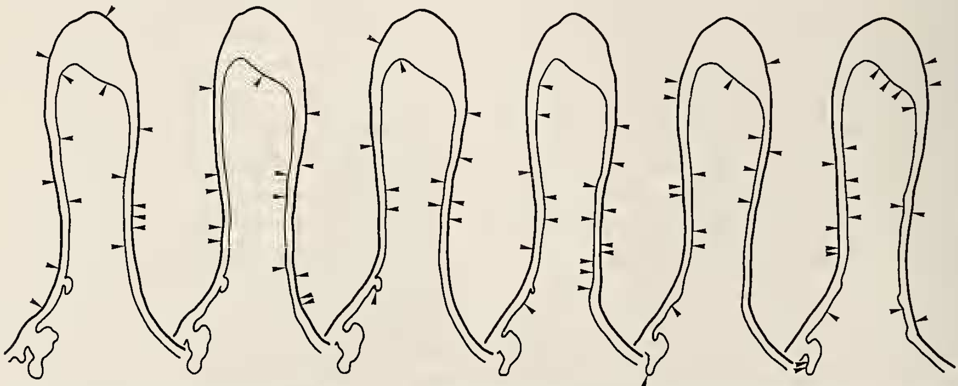

Fig. 2. Distribution pattern of dividing cells of a growing bud in *P. misakiensis.* The specimen was treated with 1mM colchicine for 12 hr before fixation in order to augment mitotic figures. The outer and inner epithelia (oe, ie) were drawn with the aid of camera lucida. Arrowhead shows a single dividing cell. (Kawamura and Nakauchi, unpubl.).

2.5 in *Botrylloides simodensis* and 0.15 in *Polyandrocarpa misakiensis* (unpubl. data), and the cell cycle time was estimated as 170–200 hr in the latter species [25].

Figure 3 shows the basic strategy of organogenesis in botryllid buds. Most tissues and organs other than the epidermis form from the inner vesicle and/or mesenchymal cells, so-called lymphocytes [26–28] or hemoblasts [29] (Fig. 4) (For convenience' sake, the nomenclature is standardized as hemoblast in this article). Thus, the inner epithelium and hemoblasts have been regarded as epithelial and hemocoelomic stem cells, respectively.

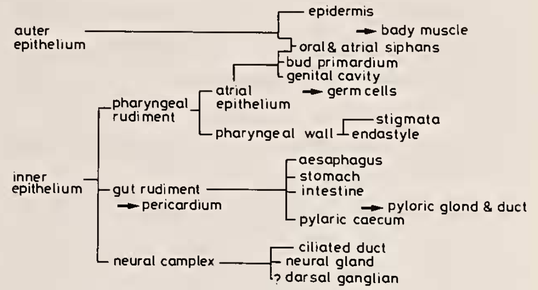

Fig. 3. Basic strategy of bud organogenesis in *Symplegma reptans*. Arrows show that constituent cells of the organ rudiments originate from hemoblasts. Adpted from Kawamura and Nakauchi [16].

Fig. 4. Behaviors of epithelial and hemocoelomic stem cells during blastogenesis of botryllid and polystyelid ascidians. (A)–(C), *P. misakiensis*. (D), *Botrylloides simodensis*. (E) (F), *Botryllus primigenus*. (A) Squamous inner epithelium (ie) of a growing bud. Bar, 10 μm. (B) Multi-layered cells of the presumptive gut domain, a 1.5-day bud, treated with 1mM colchicine. A part of dividing cells (arrowheads) are derived from hemoblasts. Bar, 25 μm. (C) The gut rudiment (g), a 2-day bud, treated with 1 mM colchicine. Bar, 25 μm. (D) Whole mount of the vascular bud (vb). Bar, 100 μm. (E) A mass of hemoblasts (arrow) associated with the wall of test vessel (tv). Bar, 25 μm. (F) The vascular bud. Its outer and inner epithelia (oe, ie) are derived from the test vessel and aggregated hemoblasts, respectively. Bar, 25 μm. (Kawamura and Nakauchi, unpubl.).

The vascular budding is the most typical example showing that hemoblasts are totipotent stem cells (Fig. 4D-F) [26, 27]. Those stem cells are characterized by a well-developed nucleolus in the large nucleus and by the basophilic cytoplasm filled with polysome [29].

The pharyngeal rudiment, gut rudiment, endostyle and neural complex are the major organ rudiments formed as folds directly from the inner vesicle of a bud. The pericardium, gonad, pyloric duct and muscle cells, on the other hand, have their cellular origin in free cells in the blood. Earlier workers [11, 13, 14, 17, 30, 31] stated that the pericardium arises from the floor of the inner vesicle. Now, we are able to describe more precisely that it arises from blood cells associated with the inner vesicle [cf. 12, 32, 33]. A full account of germ cell formation has been given by Mukai and Watanabe [34]. It is unclear whether the dorsal ganglion cells are derived from delamination of the neural complex or from an aggregate of blood cells [cf. 16].

The line that connects the pharyngeal rudiment with gut rudiment represents the antero-posterior axis (Fig. 1). The dorso-ventral axis is specified by the neural complex and endostyle, and the bilateral axis by the pericardium on the right side of the body and the intestinal loop on the left side of the body. The body pattern with bilateral asymmetry is thus formed.

(2) *Morphogenetic information for primary body patterning of buds*

There has been a great deal of confusion about the relation between parent and its buds in their body axes. Berrill [11, 35, 36] insists that in *Botryllus schlosseri* both the antero-posterior and bilateral axes coincide with those of the parental animal. Sabbadin *et al.* [37] drew an opposite conclusion that bud polarity depends on the vascularization; that is to say, the primary vessel connecting the bud with a common test vessel system is essential for the determination of bud's posterior end and the secondary vessel for the determination of ventral side. Izzard [12] described correctly that the bud axis bears a relationship to the parental axis at the skewing stage of bud hemisphere. Kawamura and Watanabe [38] have shown that in botryllid and polystyelid ascidians bud polarities are always influenced by the parental polarities, the phenomena referred to as parental lateral effect. They assumed that the parental lateral effect accounts for the manner by which the bilaterally asymmetric body pattern such as *situs inversus viscerum* is transmitted through asexual reproduction. In fact, bud grafts that suffered the opposite lateral effect as to antero-posterior axis converted the type of bilateral asymmetry via polarity reversal [39], confirming the assumption mentioned above.

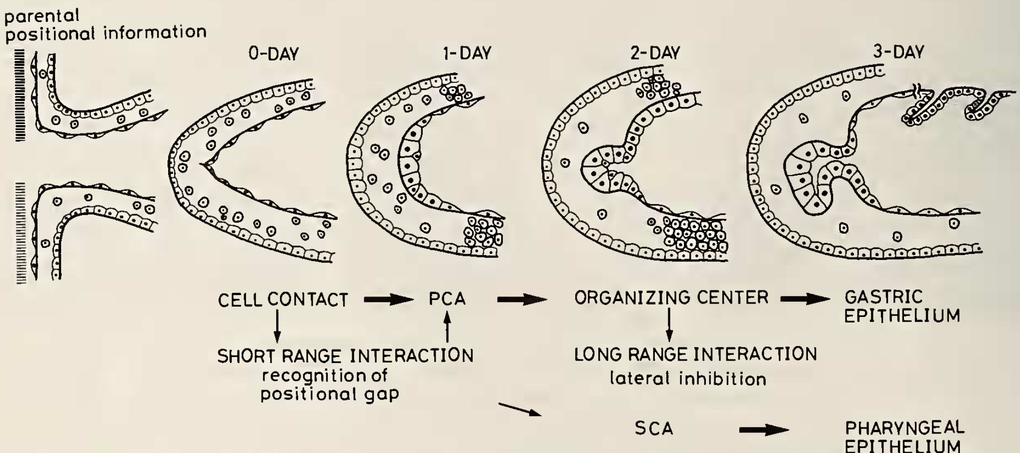

FIG. 5. A scheme of cell interactions for the position specification of gut and pharyngeal rudiments. Only epithelial cells are considered. Thick arrows show a flow of histological changes. Thin arrows show our speculation based on the results of surgical operations. PCA, primary cell activation; SCA, secondary cell activation.

Chimeric zooid analysis has shown that in *P. misakiensis* the antero-posterior axis of a bud is determined with the aid of parental positional information [40, 41]. As already mentioned, the anterior and posterior ends of a bud are characterized by the pharyngeal and gut rudiments, respec-

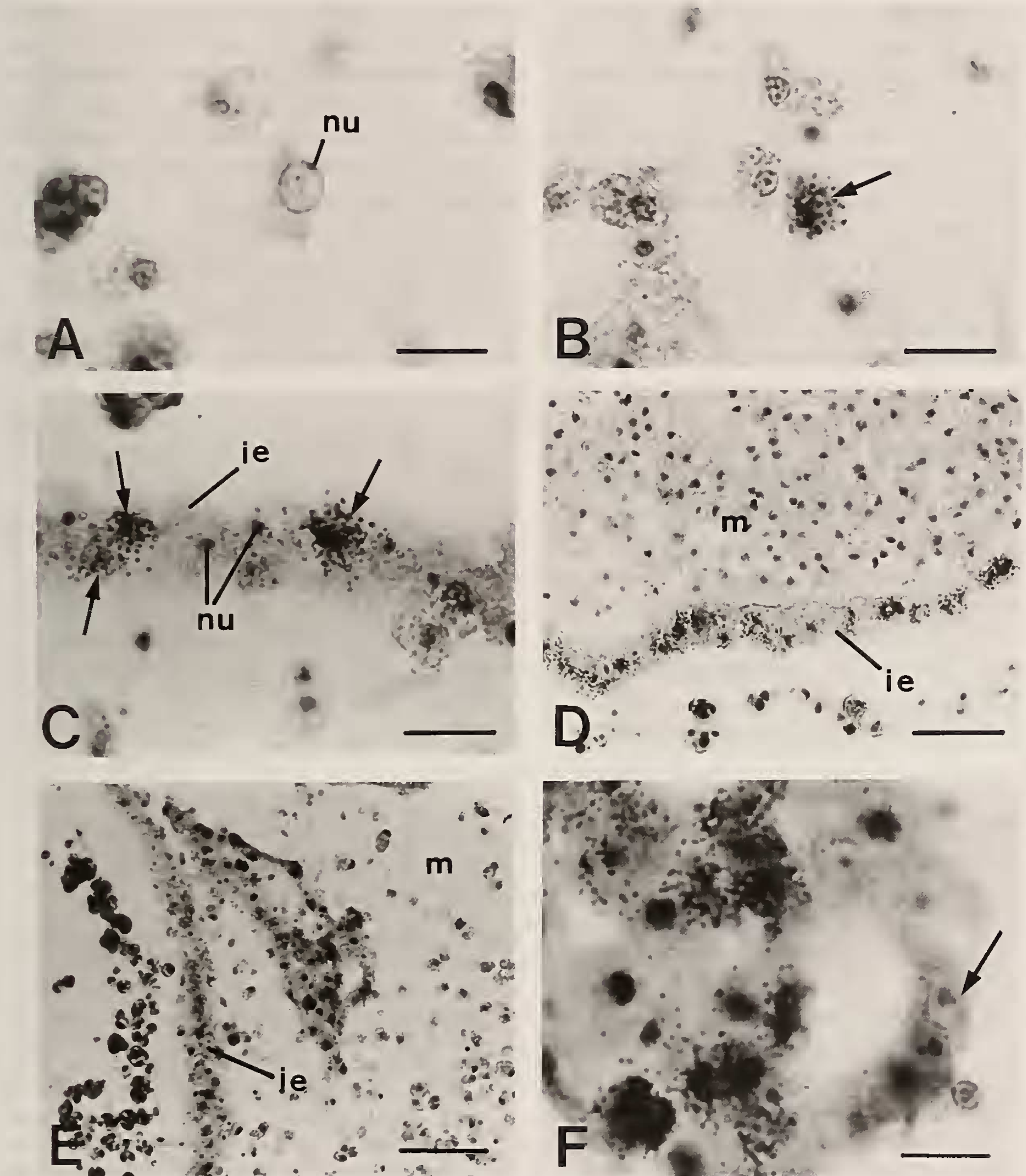

FIG. 6. [^{3}H] Thymidine-incorporating cells of a two-day-developing bud in *P. misakiensis*, 20-min pulse labeling. (A) Hemoblast with a large nucleolus (nu). (B) Nuclear grains of the hemoblast (arrow). (C) Inner epithelial cells (ie) with a large nucleolus (nu) of the presumptive gut domain. Arrows show labeled cells. (D) The presumptive pharyngeal domain. No grains were found in the mesenchymal space (m). (E) Aggregating hemoblasts in association with the presumptive gut domain of inner epithelium (ie) in the mesenchymal space (m). (F) Higher magnification of the cell aggregate. Arrow shows the epithelial transformation of hemoblasts. Bars of (D) and (E) indicate 25 μm and 50 μm, respectively. Other bars indicate 10 μm. From Kawamura *et al.*, [25].

tively. The gut rudiment can be specified autonomously at the site of discontinuity of positional information [41, 42]. If an additional gap of positional values is given experimentally to a bud, an additional gut rudiment forms. On the other hand, the pharyngeal rudiment forms in the lateral wall with lower (more anterior) positional values, thus the antero-poterior axis being skewed toward the parental anterior end [41]. The resultant antero-posterior fate map of a *Polyandrocarpa* bud (Fig. 1) was consistent with Izzard's observation made on *Botryllus,* mentioned above.

(3) *Effect of positional information on the behavior of stem cells*

In *P. misakiensis,* the inner epithelium changes cell shape from squamous to coboidal through multilayered spherical form at the morphogenesis domain (Figs. 4A-C, 5) [33, 43]. The epithelial cells began to incorporate [^{3}H]thymidine 36–42 hr after the onset of bud development and enter cell cycling with the cycle time of about 12 hr ($G_1=2.3$, $S=5.0$, $G_2=4.9$, $M=0.3$) (Fig. 6) [25]. The cell activation takes place in two steps: the primary activation at the presumptive gut domain, and the secondary activation at the pharyngeal domain (Fig. 5) [33, 43].

Hemoblasts underwent blasto-transformation and aggregated in the mesenchymal space to form organ rudiments or they infiltrate into the inner epithelium (Fig. 6), which seemed to contribute to the recruitment of undifferentiated cells to the inner epithelium. The aggregation and epithelial transformation of hemoblasts could not be blocked by aphidicolin (1–10 μg/ml), an inhibitor of DNA polymerase α, by α-amanitin (10 μg/ml), an inhibitor of RNA polymerase II, and by 1 mM colchicine, an anti-mitotic drug, and it was influenced partially by puromycin (200 μg/ml), an inhibitor of protein systhesis [43, 44 and unpubl. data].

Those behaviors of epithelial and hemocoelomic stem cells could be induced additionally by surgery in which a host bud is sandwiched between two grafts with higher positional values [43]. Such a sandwiched bud formed the gut rudiment at respective sites with positional disparities, usually resulting in biposterior zooids. The magnitude of mitosis in the bud was in parallel with the degree of positional information gap between two bud pieces juxtaposed (Fig. 7) [43]. Our result is consistent with the prediction of the polar coordinate model for pattern formation [45, 46], which predicts that a disparity of positional values triggers cell division. In conclusion, we suggest that short-range cell interactions based on parental positional information influences cell cycling and aggregation of stem cells, and consequently the position specification of antero-posterior body patterning of *Polyandrocarpa* buds.

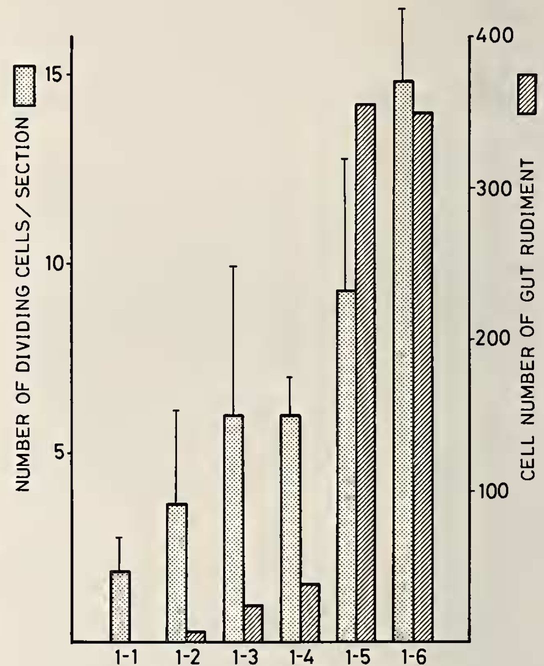

Fig. 7. Effect of positional information gaps on the mitotic activity and size of gut rudiment. The proximo-distal half of a bud derived from the parental sector 1 was juxtaposed with that of a bud from sector 1 to sector 6 (As for the operation procedure, see [40–43]). The buds were allowed to develop for two days and treated with 1mM colchicine for 12 hours before fixation. The result shows that the mitotic activity of the gut domain depends on the magnitude of positional information gaps. It should be also noted that the larger the positional information gap becomes, the larger gut rudiment is established, suggesting that a gap of positional values enhances organogenesis. Bars indicate the limit of 95% confidence. (Kawamura and Nakauchi, unpubl.).

HOMEOSTATIC TISSUE INTERACTIONS IN BLASTOGENESIS

One of the most prominent features of palleal budding is that the developmental phase of a bud is clearly separated from its growth phase. In *P. misakiensis,* a bud enters the developmental phase with ease, irrespective of its age, by extirpating it from the parental animal [23, 39, 47]. The isolated bud piece went on growing without morphogenesis if it was grafted again onto the adult mantle wall [39]. In another experiment, a growing bud was cut at both the proximal and distal extremities and grafted with rotated proximal-distal axis into the parental mantle wall [39, 48]. Unlike the former experiment, the original proximal end that is the morphogenesis domain [23] is now at the distal free surface and discontinuous with the parental tissues. In this case, too, the bud did not enter the developmental phase. The result strongly suggested that bud development might be triggered not by a mechanical stimulus but by a release from a humoral factor derived from the parent.

There has been a classical idea that tissue homeostasis is controlled by the balance between growth-stimulating and inhibiting signals. Epidermal growth factor [49] and liver-specific tripeptide [50] are well-known tissue-specific stimularoty factors. The head activator of hydra is a peptide consisting of 11 amino acids [51]. It acts as an autocrine growth factor localized in nerve cells [52]. They are thought to be released locally after injury. On the other hand, little is known about the inhibitory factors named chalone. According to Bullough and Laurence (cited by [5]), epidermal cells normally produce a chalone that restricts their own rate of proliferation by G_1 arrest. Liver-specific chalones have been reported [53–55], although their molecular nature and dynamic aspects during liver regeneration are uncertain. In *P. misakiensis,* alcohol extracts of parental colonies contain a few cell division regulators of low molecular weight (Kawamura and Fujiwara, in preparation). Works on their *in vivo* function are now in progress.

Homeostatic integration is also found to govern bud development in some botryllid and polystyelid ascidians. Nakauchi *et al.* [19] observed that when an isolated stolonic bud of *Polyzoa vesiculiphora* is ligated in the middle region, it develops into two functional animals, but that it becomes a single animal if the ligation is for primary 30 hr. They interpreted the result as indicating that during this time period cell-cell interacations take place in order to establish a single organization center. In sandwich buds of *P. misakiensis,* the induction ratio of double guts depended on the distance between two positional information gaps [41]. The result suggested two possibilities. In one, the distance might reflect merely the cell number required for the formation of gut rudiment. Alternatively, it might reflect a long-range cell signalling for the formation of a single gut rudiment.

Double-half bud experiments using *P. misakiensis* gave more direct evidence for homeostatic tissue interactions, or lateral inhibition, during bud development [42]. A presumptive posterior bud half failed to form the posterior end irrespective of its positional values if it was combined with a pre-determined bud half. The result strongly suggests that the posterior end, once established, blocks an additional formation of the homogenous structure.

According to the definition of Huxley and de Beer [56], the posterior end is the dominant region of *Polyandrocarpa* buds: its formation is autonomous, it is first to be established and it blocks the formation of the same structure. In regenerating hydras, both head and foot are the dominant region [6–8, 57–59]. Several signalling molecules have been extracted from hydras [60, 61]. Inhibitory signalling is known in more details at the cellular level in some embryos. In the equivalence group of *C. elegans* embryos, one of equivalent cells adopts a primary fate, while the other cells adopt a secondary fate [62]. Cell-cell interaction takes place between these cells in which the cell adopting the primary fate prevents the other cell from also adopting this fate [63]. The gene product of *lin* 12 is necessary for such cell signalling [64, 65]. *lin* 12 is homologous to a *Drosophila* gene, *Notch,* that has a sequence homology with epidermal growth factor [66].

One of the interesting problems of *Polyandrocarpa* buds is whether or not the inhibition of an additional gut formation accompanies the suppres-

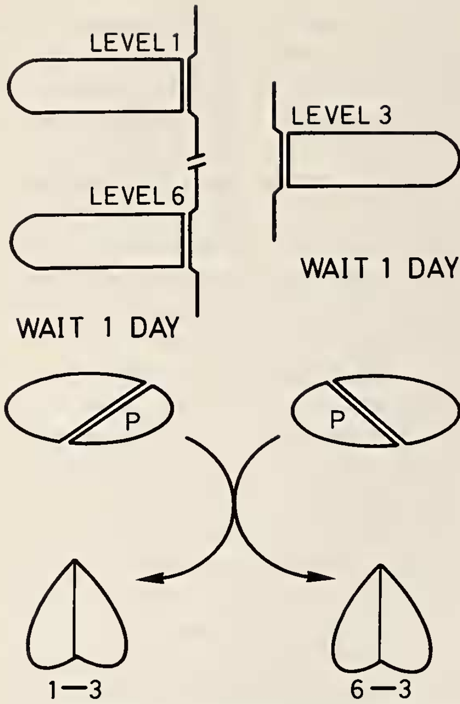

Fig. 8. Procedure of construction of double-posterior buds. Buds derived from the parental sector (level) 1, 3 or 6 were allowed to develop for one day, and then divided into the presumptive anterior and posterior halves. Two kinds of double-posterior (1–3 and 3–6) buds were constructed and fixed two days later, preceded by the treatment of 1 mM colchicine for the last 12 hr. The gut formation and the number of mitotic figures were examined histologically. Special attention was paid to how the behavior of stem cells in level 3 domain was influenced by the bud half of level 1 or level 6 juxtaposed.

sion of cell cycling and aggregation of stem cells. One-day posterior fragment taken from level 3 was combined with either that taken from level 1 (the lowest positional value) or that taken from level 6 (the highest positional value) (Fig. 8). Those 1–3 and 3–6 double-half buds were allowed to develop for further two days and examined histologically with reference to the behaviors of stem cells. In those double-half buds, there was a difference in the number of dividing cells between the same level halves (level 3) combined with different levels (level 1 or 6) (Table 1). The result showed that the cell cycling was influenced by bud tissues juxtaposed, and strongly suggested that pattern regulation of *Polyandrocarpa* buds accompanies the inhibition of cell division cycle. On the other hand, aggregation of hemoblasts could be seen irrespective of positional levels of bud halves juxtaposed (unpubl. data).

DEVELOPMENTALLY REGULATED GRANULE EXOCYTOSIS IN RELATION TO HOMEOSTATIC INTEGRATION

Recently, a galactose-specific, 14 kDa lectin has been purified from *P. misakiensis* [67]. Its amino acid sequence shows 20–30% homology with those of fly [68], barnacle [69], sea urchin [70], and several vertebrate lectins that belong to C-type lectin [71]. The C-type lectin domain has also been found in cell adhesion molecule associated with inflammation [72], and lymphocyte homing recep-

Table 1. Pattern regulation of double-posterior buds

Series	combination #	No. of operation	Gut formation		Average number of dividing cells/section	
			level 1 or 6	level 3	level 1 or 6	level 3
I	1–3	7	0	4	2.1±1.3 ##	4.5±1.3
	6–3	7	6	0	4.7±2.1	1.7±0.8
II	1–3	8	2	7	N.D. ###	15.2±5.6
	6–3	8	8	1	19.1±4.8	8.2±4.9
III	1–3	12	3	12	N.D.	7.6±1.9
	6–3	5	5	1	9.4±5.8	4.3±1.1

As for the experimental procedure, see Fig. 8.
Mean±standard deviation.
Not determined.
(unpubl. data of Kawamura and Nakauchi).

tor [73]. Although the relationship with these findings is unclear, the N-terminal 35 residues of the *Polyandrocarpa* lectin show 40% homology with the partial sequence of the variable region of the Ig $\varkappa$-chain [67].

Immunocytochemical studies have shown that the *Polyandrocarpa* lectin is induced specifically in budding (Kawamura *et al.*, in preparation). Polyclonal anti-lectin antibody reacted with granules of the bud's inner epithelium at the earliest stage of bud formation (Fig. 9A), while it did not react with the surrounding parental tissues. About one day after the extirpation, the inner epithelial cells facing the cut surface began to secrete the lectin-positive granules in the mesenchymal space. Consequently, lectin-positive extracellular matrix (ECM) developed in a dendritic pattern within two days of bud development (Fig. 9B). Because of the strong affinity of the lectin with hemoblasts, we suggest that the ECM containing lectin domain facilitates the aggregation of hemoblasts during bud development.

In mammals, granule membrane protein 140 (GMP-140) has been identified as an integral mem-

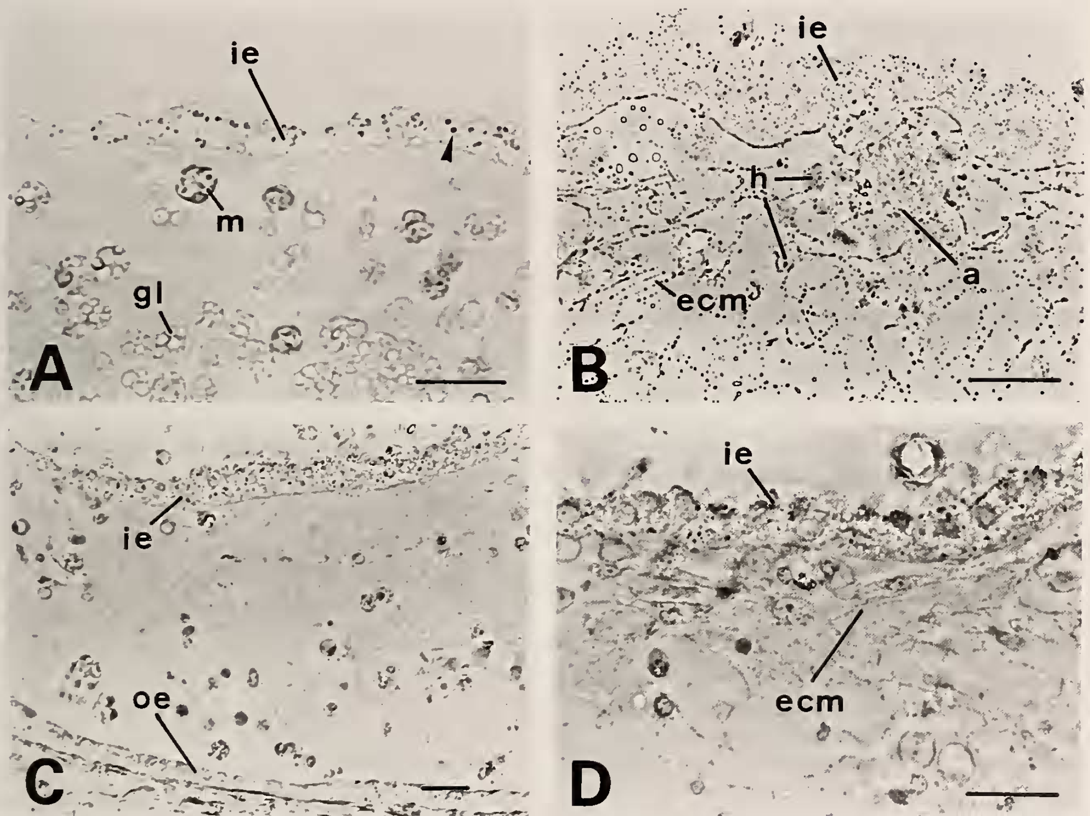

FIG. 9. Immunocytochemistry of 14 kDa *Polyandrocarpa* lectin. Sections were reacted with rabbit anti-lectin polyclonal antibody and goat secondary antibody labeled with peroxydase, diaminobenzidine staining. (A) Growing bud. Granules (arrow) of the inner epithelium (ie) were lectin-positive. Staining of morula cells (m) was derived from endogenous peroxydase. Granulalr leukocytes (gl) were lectin-negative. (B) The gut domain of a 2-day-old bud. The extracellular matrix (ecm) was lectin-positive. Hemoblasts (h) were forming a cell aggregate (a). Note that the outline of aggregating cells is not reacted with the antibody. (C) A 2-day bud in the presence of 1 μM indomethacin, the proximal end. Note that there is no extracellular matrix in the mesenchymal space between the outer and inner epithelia (oe, ie). (D) The proximal end of a 2-day bud in the presence of 1 μM indomethacin and 15nM prostaglandin $F_{2\alpha}$. Lectin-positive extracellular matrix (ecm) appeared, although developed poorly, beneath the inner epithelium (ie). Bars, 25 μm. (Kawamura, unpubl.).

brane protein found in secretory granules of platelets and endothelial cells [74]. It contains soluble and membrane-bound forms, both of which have the C-type lectin domain at the N-terminus [72]. At inflammatory and coagulation responses, the GMP-140 is secreted or expressed on the plasma membrane soon after cell activation by mediators such as thrombin, and is involved in *in situ* platelet aggregation and leukocyte-endothelial cell adhesion [e.g. 75]. In *P. misakiensis,* the contact surface of aggregated hemoblasts or inner epithelial cells was not stained with anti-lectin antibodies (Fig. 8B), suggesting that the lectin does not play a role in cell adhesion.

During bud development of *P. misakiensis,* granule exocytosis was often observed at the morphogenesis domain. For example, granular leukocytes almost disappeared owing to autolysis and phagocytosis following degranulation [33, 48]. The morula cell, a kind of vacuolated blood cells in ascidians, showed an endogenous peroxydase activity (Kawamura, unpubl. data). They also showed membrane-bound NADPH oxydase activity, an indicator of O_2^- production, in developing buds (Kawamura, unpubl. data). These results suggest that superoxide and its derivatives such as O_2^- and H_2O_2 are secreted in the mesenchymal space during bud development. They may play a role in antimicrobial mechanism after injury.

It is reasonable to assume that the granules to be secreted may contain some biologically active substances such as cell cycle regulators and cell adhesion modulators. We suggest that pattern regulation of ascidian buds is realized partly by the exocytosis of so-called autacoids in spatiotemporally regulateld manner. Our hypothesis explains how morphogenesis takes place in the presence of inhibitors of RNA and protein synthesis, as methioed before.

Recently, we have found that in *P. misakiensis* the exocytosis and other various phenomena of bud development can be blocked by indomethacin [76]. Indomethacin is a non-steroid, anti-inflammatory drag that inhibits specifically the enzyme activity of cyclooxygenase mediating the production of endoperoxides such as prostaglandin G_2 (PGG_2) and PGH_2 from arachidonic acid. It blocked bud development of *P. misakiensis* including cell division cycle and aggregation of stem cells at the concentration of 0.4 μM [76]. In this condition, neither lectin-positive granules in the inner epithelium were secreted into the mesenchymal space nor the ECM developed (Fig. 8C). Interestingly, in the presence of indomethacin the ECM as well as hemoblast aggregation appeared by adding 15 nM $PGF_{2}\alpha$, a downstream product of arachidonic acid cascade (Fig. 8D). In general, the exocytosis requires the increase in cytosolic concentration of Ca^{2+}. At fertilization, the cortical granule (vesicle) exocytosis of eggs and the acrosome reaction of sperm can be induced by Ca^{2+} ionophore [e.g. 77]. The calcium release is triggered normally by inositol triphosphate that is a metabolic product of membrane phospholipids [e.g. 78]. $PGF_{2\alpha}$ facilitates this metabolism of inositol phospholipids [79–81].

In *Polyandrocarpa* buds, cell cycling of epithelial stem cells was recovered partially by adding 15 nM PGE_2 in the presence of indomethacin [76]. Recently, PGE_2 has been shown to play a role in DNA synthesis of sponge cells at the late stage of cell aggregation [82]. This eicosanoid has also been found in ovaries of the termite queen, *Macrotermes subhyalinus* [83], although its biological function is uncertain. Another pathway of arachidonic acid cascade mediated by lipoxygenase is known in some invertebrate oocytes such as starfish [84] and ascidian [85]. In starfish oocyte maturation, 8-hydroxy-eicosateraenoic acid seems to play a role in the transduction of the 1-methyladenine message at the plasma membrane level [84].

CONCLUSION

The gut rudiment is a dominant fate of epithelial and hemocoelomic stem cell lines during bud development of *P. misakiensis.* The primary signalling for the dominant fate is given by short-range interactions between cells with different values of parental positional information. Although there is no evidence, at present, about the molecular nature of parental positional information, we assume that the primary signalling would induce granule exocytosis. Then, secreted substances with biological activity act as the secondary signal, for exam-

ple, in order for stem cells to enter cell cycling. On the basis of our assumption, the homeostatic integration for body patterning in ascidian buds may be involved in the control of trans-membrane signalling required for the exocytosis.

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